Transdermal Delivery of Insulin to Alloxan-Diabetic Rabbits by Ultrasound Exposure

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INTRODUCTION

Transdermal delivery systems have been widely accepted clinically for administration of systemic drugs such as scopolamine, clonidine, and nitroglycerin. The transdermal delivery of drugs bypasses hepatic "first-pass" metabolism and maintains a steady drug concentration. However, the stratum corneum functions as a barrier for most drugs. Intensive research to increase drug penetration resulted in the use of iontophoresis and phonophoresis. Kari (1) reported transdermal delivery of insulin in diabetic rabbits by iontophoresis. However, drug permeability was influenced by pH, thus preventing the use of this method for long durations. Transdermal drug delivery by ultrasound has been reported in 1954 by Fellinger (2), who treated polyarthritis with ultrasound and hydrocortisone. Griffin *et al.* (3) studied the effects of topical hydrocortisone and ultrasound by double-blind comparison. McElnay *et al.* (4) reported transdermal absorption of fluocinolone acetonide by exposure to ultrasound. Although these studies show significant differences in drug absorption between ultrasound-treated groups and controls, the effects of the ultrasound were smaller than expected (5,6), possibly because of the minute amount of drug that penetrates the skin barrier by this method. In most studies the drug was prepared as a cream or gel for administration to the skin for anesthetic purposes.

Transdermal delivery by ultrasound was reported with D-mannitol, inulin, and physostigmine in rats and guinea pigs (7). Ultrasound shortened the lag time for these drugs to penetrate the skin. Brucks *et al.* (8) succeeded in ultrasound penetration of ibuprofen in human epidermis *in vitro*. We previously reported transdermal delivery of insulin by ultrasound in hairless mouse (9,10). These reports suggested new aspects of the mechanism of ultrasound enhancing effects other than "massage." Thermal and nonthermal effects of ultrasound (cavitation, radiation pressure, and acoustic microstreaming) can alter the permeability of drug through the skin.

In the present study, a small piezo electric element with a drug reservoir was devised to deliver insulin through the skin for induction of systemic blood glucose changes in diabetic rabbits. Ultrasound was delivered over relatively longer periods with aqueous drug forms instead of the cream or gel used in previous reports.

MATERIALS AND METHODS

Japanese white rabbits weighing 2–3 kg were obtained from a local supplier. The hair of the back and abdomen was clipped with an electric shaver. Special care was taken not to abrade the skin during this procedure. The skin was closely examined after 48 hr. The rabbits were then selected for the experiments if the clipped area was in the resting phase of the hair growth cycle to avoid additional clipping and abrasion. Any sign of skin disruption or rash was unacceptable. The bare surface area was no smaller than 7 × 7 cm. Approximately 70% of the rabbits were eliminated at this stage. The selected rabbits were then intravenously injected 125 mg/kg of alloxan (Nacalai Tesque Inc., Kyoto, Japan) as a 10% solution in sterile water. Fasted blood glucose concentration was assayed 2 days after this treatment. Animals with blood glucose levels over 200 mg/dl were defined as alloxan-induced diabetic rabbits. The animals had free access to food and water after alloxaniztion.

On the day of the experiment, the rabbits were anesthetized by peritoneal injection of 25 mg/kg pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago) after overnight fasting. The bare area of the skin to be used was gently washed with warm tap water and dried naturally. Ultrasound was exposed to the skin by a piezo electric oscillating element (Kyocera Corp., Japan) attached to a metal cup (3.5 cm in diameter; 2 mm deep). Three milliliters of 40 U/ml neutral purified pork insulin (Insulin Novo Actrapid MC, Novo Industry, Denmark) was filled inside the space between the skin and the ultrasonic element (Fig. 1). The skin area in direct contact with the drug was 7 cm^2. The ultrasound element and drug were sealed by adhesive tape (Nichiban Corp., Japan) to prevent leaks and contamination. All air pockets were driven out of the chamber. Ultrasound audio generator LAG-26 (Leader Electronics Corp., Japan) and power amplifier Type 2713 (Bruel & Kjaer, Denmark) were used to drive the ultrasound element. The frequency was 105 kHz and the ultrasound energy was 5000 Pa, with measurement by a hydrophone (8103 Hydrophone, 2635 Bruel & Kjaer). Ultrasound was delivered in a pulse for a duration of 5 sec at 5-sec intervals. The drug and ultrasound element were removed from the skin after a 90-min application. The device was kept at 4°C throughout this period to prevent drug degradation. The skin was closely examined after ultrasound exposure for any signs of injury. Skin biopsy samples were stained with hematoxylin and eosin after the experiments. Blood samples (1 ml) were taken from the ear vein at 0, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 hr after beginning the experiments. Blood glucose concentrations were measured using a glucose oxidase (GOD) method (Wakoujunyaku Corp., Japan). Serum insulin concentrations were measured with an Insulin Enzyme Immunoassay Dainabot Kit (Abbot Laboratory, Chicago). For control experiments, the drug and ultrasound element were applied as described previously with the power generator off. The measurement of blood samples was identical to that in the main tests.

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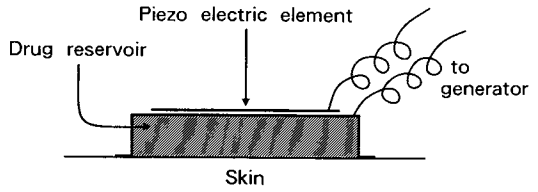

Fig. 1. Scheme of the ultrasound device and drug.

The blood glucose level and insulin concentration were compared with controls at each time point using Student's t test ($P < 0.05$).

RESULTS

Figure 2 shows the changes in percentage of the initial blood glucose level during the experiments. The glucose level of the rabbits exposed to insulin and ultrasound gradually decreased to 85.8 ± 8.8% (mean ± SD) at the end point of ultrasound exposure and reached a minimum of 58.8 ± 13.4% 3.5 hr after beginning the ultrasound exposure. Blood glucose level eventually returned to the initial level (94 ± 12.6%) by 6.5 hr. On the other hand, the glucose level in control experiments was slightly elevated or remained unchanged. Glucose levels were significantly different ($P < 0.05$) between the main and the control experiments except at 6.5 hr.

The mean maximum plasma insulin concentration was elevated in ultrasound-exposed animals compared to control animals (Table I). The mean minimal blood glucose level showed significant differences between ultrasound-exposed and unexposed animals. Although all minimum blood glucose levels were detected between 2.5 and 3.5 hr after starting the experiment, the maximum plasma insulin concentration occurred at various time points. The maximum plasma insulin concentration, in some cases, was detected before or after minimum blood glucose. Figure 3 shows a case where the insulin concentration increased from 11.8 to 45.6 μU/ml during ultrasound exposure and slowly returned to the initial level after ultrasound was discontinued. Glucose concentration, on the other hand, decreased from 326 mg/dl to a minimum level (151 mg/dl) 2 hr after insulin reached its peak level. Glucose returned to the initial level in the next 4 hr.

Table I. Blood Glucose Level and Plasma Insulin Concentration Comparison of Treated and Untreated Groups (Mean ± SD)

	Blood glucose level (mg/dl)		Plasma insulin concentration (μU/ml)	
	Initial[a]	Minimum[b]	Initial[a]	Maximum[b]
Control ($N = 5$)	355 ± 67	342 ± 66	6.1 ± 6.4	15.3 ± 14.1
Ultrasound ($N = 9$)	313 ± 66	195 ± 54	8.4 ± 5	120 ± 110

[a] Value obtained just before applying ultrasound.
[b] Lowest and highest values obtained during the course of the experiment.

Examination of the skin by the naked eye after exposure to insulin and ultrasound disclosed no sign of burns or erythema. Histological findings by microscope revealed no inflammation or destruction of tissue. The stratum corneum was intact.

DISCUSSION

Transdermal insulin absorption is limited because of its high molecular weight, short half-life, and chemical instability. Frequent hypodermic injection of insulin is the only practical method for diabetic patients. In this study, a device with a piezo electric element was used to deliver insulin through the skin in alloxan-induced diabetic rabbits by ultrasound. Insulin and ultrasound exposure to the skin resulted in an elevation of plasma insulin and a decrease in blood glucose level. Glucose levels returned to the original level after ultrasound was stopped. However, insulin alone on the skin did not show significant changes in plasma insulin or blood glucose.

Previous reports (11) of transdermal delivery by ultrasound had applied commercially available ultrasound devices usually used for physical therapy. The ultrasound energy ranged from 0 to 3 W/cm^2 and the frequency was usually between 20 kHz and 10 MHz. Although the transducers were compact, application to the skin were often hand-held.

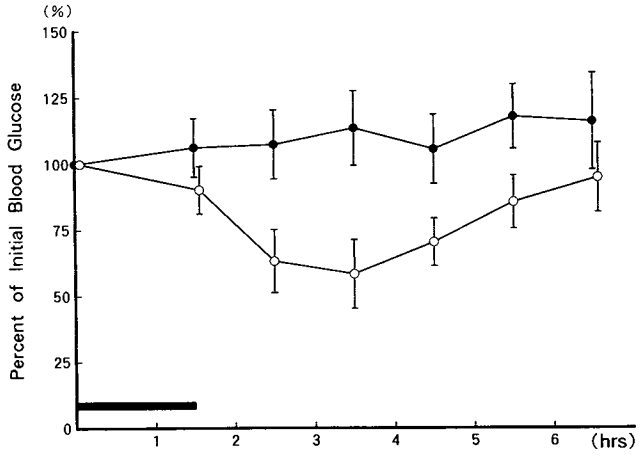

Fig. 2. Open circles indicate blood glucose level after ultrasound exposure from 0 to 1.5 hr. Filled circles indicate control (mean ± SD).

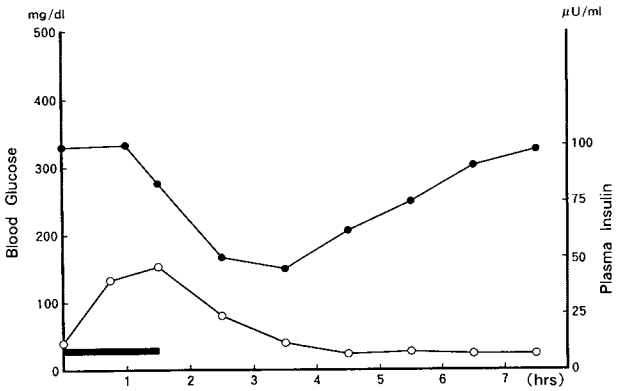

Fig. 3. Filled circles indicate blood glucose level and open circles show plasma insulin concentration.

Exposure duration was considerably short. Levy *et al.* (7) and Benson *et al.* (6) reported an exposure time of 5 min. In the present study, a relatively low-energy ultrasound enabled the miniaturization of the transducer. The ultrasound device was placed and fixed with the drug reservoir between the skin and the ultrasound element, making it more practical and convenient for longer periods of ultrasound exposures. The aqueous solution of the drug kept the availability of the drug to the skin at a constant rate compared to cream or gel preparations. In addition, the device was completely sealed to prevent leaks or evaporation of the drug thus stabilizing ultrasound transfer to the skin.

There was a marked lowering of blood glucose caused by ultrasound exposure to the skin with insulin. Blood glucose levels continued to decrease after ultrasound and insulin exposure were stopped. Similar results have been reported with iontophoresis of insulin (1). Levy *et al.* reported elevation of drug levels after ultrasound exposure (7). It was speculated that insulin accumulation in the stratum corneum and slow washout at the dermoepidermal junction may result in the delay of systemic reaction of blood glucose. However, Brucks *et al.* (8) reported no lag time in human epidermis during ultrasound exposure. Figure 3 demonstrates a plasma insulin concentration increase during ultrasound exposure and a decrease afterward, without the lag time. However, these results were not always reproducible. Plasma concentrations of insulin were significantly elevated by ultrasound exposure, not always in correlation with peak glucose levels. Previous experiments on transdermal delivery of insulin have also shown discrepancies in plasma insulin concentrations (2). The short biological half-life and interanimal variation of metabolic breakdown of insulin may account for these discrepancies.

The mechanism of ultrasound transdermal delivery of drugs is unclear. The barrier function of the skin is located in the stratum corneum of the epidermis, which prevents most drugs from absorption into the body. The skin also consists of hair follicles, sweat ducts, apocrine glands, and eccrine glands. These sites may be routes for drug penetration. It is postulated that ultrasound enhanced drug permeability through any of these routes.

Further investigations must be carried out to assess the relationship between ultrasound power and drug permeability. The solution of the drug, frequency, and duration of exposure may affect enhancement.

In conclusion, ultrasound exposure with insulin to the skin induced sufficient effects of blood glucose lowering in diabetic rabbits. This result suggests an alternative method of insulin delivery for control of blood glucose level in diabetic patients.

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